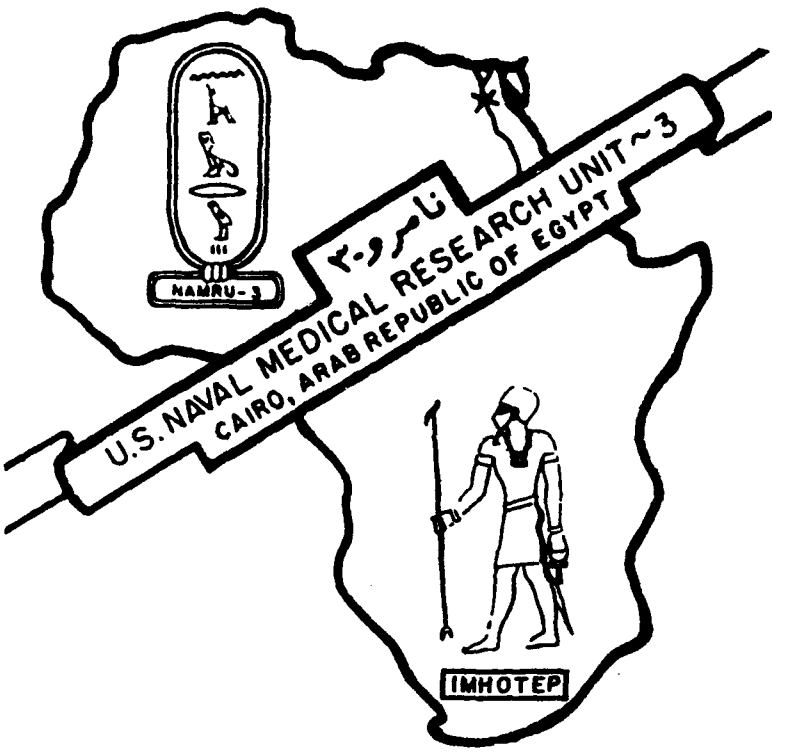
12



TECHNICAL / SCIENTIFIC REPORT

1295

30/82

DIAGNOSIS OF HUMAN BRUCELLOSIS WITH ELISA

By

John E. Sippel, Nabil A. El Masry, Zohair Farid

U. S. NAVAL MEDICAL RESEARCH UNIT No. 3
(CAIRO, ARAB REPUBLIC OF EGYPT)
FPO NEW YORK 09527

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER NAMRU-3-TR. 30/82	2. GOVT ACCESSION NO. AD-A122 958	3. RECIPIENT'S CATALOG NUMBER
4. TITLE *(and Subtitle)* DIAGNOSIS OF HUMAN BRUCELLOSIS WITH ELISA		5. TYPE OF REPORT & PERIOD COVERED Technical/Scientific Report
		6. PERFORMING ORG. REPORT NUMBER ACC-1295
7. AUTHOR(s) John E. Sippel, Nabil A. El Masry, Zohair Farid		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Naval Medical Research Unit No.3 FPO New York 09527		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS M0095-PN-002-5020
11. CONTROLLING OFFICE NAME AND ADDRESS Naval Medical Research and Development Command National Naval Medical Center Bethesda, MD 20814		12. REPORT DATE 3 July 1982 13. NUMBER OF PAGES 3
14. MONITORING AGENCY NAME & ADDRESS*(if different from Controlling Office)*		15. SECURITY CLASS. *(of this report)* Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

Distribution of this report is unlimited

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

Published in: The Lancet 2:19-21, 1982

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

Brucellosis
ELISA tests
Acute and chronic phases
Direct agglutination test
Patients and methods

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

ELISA tests for total (IgG+IgM+IgA), IgG, and IgM anti-Brucella antibodies, which utilised only commercially available reagents, were used to diagnose human brucellosis. Assays for total antibodies in sera from 22 patients with confirmed acute brucellosis, 1 patient with probable acute brucellosis, and 3 patients with probable chronic brucellosis gave readings that were more than double those found in hundreds of control sera. All sera from patients with acute and chronic brucellosis had significantly elevated IgG

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE
S N 0102-LF-014-6601

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

NAMRU-3-TR. 30/82 (Contd.)

levels. Although there were a few acute patients with IgM levels only slightly higher than those of some controls, most patients with acute disease could readily be differentiated from both the non-brucellosis patients and patients with chronic brucellosis by measuring macroglobulins. Both the IgG and IgM levels in sera from acute patients persisted for at least 8 months. The results of this study show that ELISA is an excellent method for screening large populations for Brucella antibodies and for differentiation between the acute and chronic phases of the disease.

Reprinted from THE LANCET, *July 3, 1982, pp. 19–21*

DIAGNOSIS OF HUMAN BRUCELLOSIS WITH ELISA

JOHN E. SIPPEL* N. AYAD EL-MASRY
ZOHAIR FARID

U.S. Naval Medical Research Unit No. 3, Cairo, Egypt

Summary ELISA tests for total (IgG + IgM + IgA), IgG, and IgM anti-*Brucella* antibodies, which utilised only commercially available reagents, were used to diagnose human brucellosis. Assays for total antibodies in sera from 22 patients with confirmed acute brucellosis, 1 patient with probable acute brucellosis, and 3 patients with probable chronic brucellosis gave readings that were more than double those found in hundreds of control sera. All sera from patients with acute and chronic brucellosis had significantly elevated IgG levels. Although there were a few acute patients with IgM levels only slightly higher than those of some controls, most patients with acute disease could readily be differentiated from both the non-brucellosis patients and patients with chronic brucellosis by measuring macroglobulins. Both the IgG and IgM levels in sera from acute patients persisted for at least 8 months. The results of this study show that ELISA is an excellent method for screening large populations for *Brucella* antibodies and for differentiation between the acute and chronic phases of the disease.

Introduction

BRUCELLOSIS is usually diagnosed in the laboratory by means of blood culture or demonstration of elevated levels of humoral antibody. Since blood culture can take weeks and is often unsuccessful, a positive diagnosis is more frequently made serologically. Tube agglutination, the most commonly used serological method, is less than ideal because it is laborious, takes 2 days, and can be falsely negative, owing to the presence of blocking antibodies. However, it can differentiate immune classes of specific antibodies—IgM associated with acute brucellosis and IgG associated with chronic infections. This is accomplished by testing sera before and after treatment with 2-mercaptoethanol.[1]

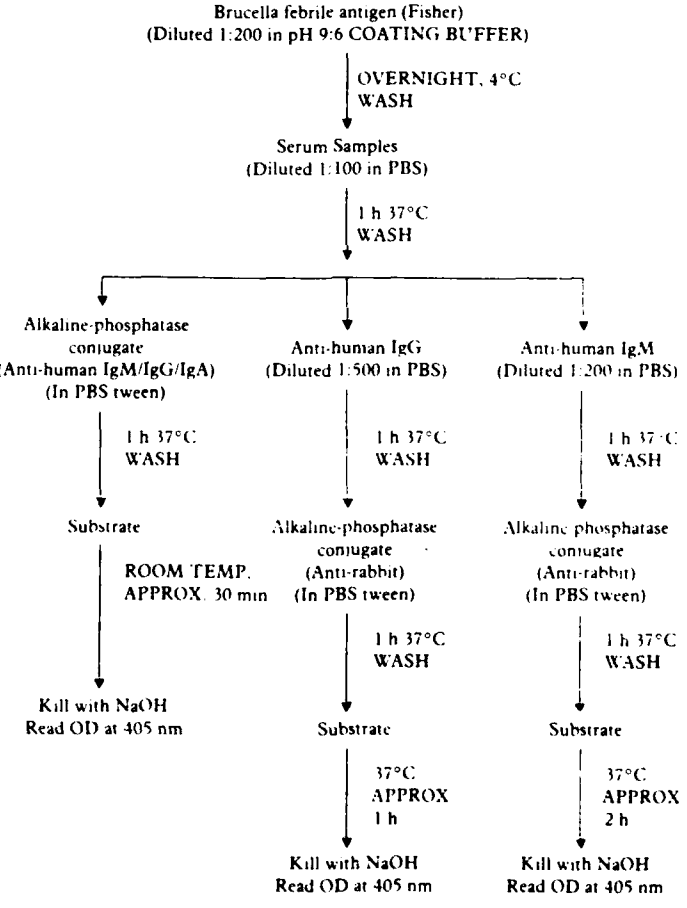
*Present address: Naval Biosciences Laboratory, Naval Supply Center, Oakland, California 94625, U.S.A.

Complement fixation, anti-human-globulin testing, and radioimmunoassay have also been used to differentiate IgM and IgG antibodies in brucellosis patients.[2-5] Results of a limited study suggested that specific immunoglobulin levels could be more simply determined with the enzyme-linked immunosorbent assay (ELISA).[6] We here extend the use of the ELISA method to measure total *Brucella* antibodies and IgG and IgM *Brucella* antibodies in 22 patients with acute brucellosis and several hundred controls in Egypt.

Patients and Methods

Patients

Sera were collected from 86 patients in the Abbassia Fever Hospital, Cairo, from January, 1980, to June, 1981. 22 of the



ELISA procedures used to measure total, IgG, and IgM anti-*Brucella* antibodies.

PBS = phosphate-buffered saline. OD = optical density.

patients had acute brucellosis as determined by positive blood culture for *Brucella melitensis* (19) or agglutination titres of at least 1:320 with a clinical presentation compatible with this disease. Sera from 324 febrile patients not suspected of having brucellosis collected in Upper Egypt and Alexandria were also included in this study.

ELISA

The methods used for measuring total (i.e., IgG + IgM + IgA) anti-*Brucella* antibodies and IgG and IgM antibodies are presented in the accompanying figure. The reagents were prepared as described by Voller et al.[7] Polystyrene microtitre plates were washed three times by flooding for 3 minutes with phosphate-buffered saline containing 0·05% 'Tween 20'. Duplicate reactions were compared visually, then the contents of the two wells were combined and transferred to tubes, and the final volume was brought to 2 ml with distilled water. Absorbence was read on a Perkin Elmer spectrophotometer model 550, distilled water being used as a standard. A positive control was included in each plate. This control was arbitrarily given an ELISA value of 10 and the other readings were adjusted accordingly.

Direct Agglutination Test

Standard slide and tube agglutination tests were carried out with febrile antigen (Fisher Scientific) according to methods recommended by the manufacturer. Tube agglutination was used except where otherwise noted.

Results

Tube agglutination titres and ELISA readings are listed in tables I and II. Sera from the 22 patients with acute brucellosis had total *Brucella* antibody ELISA readings greater than 7, whereas only 5 of the samples from the other 387 individuals had values greater than 3. Similarly, all 22 brucellosis patients had IgG readings greater than 7, whereas control sera were generally less than 5. The IgM data were more variable, with sera from 9 brucellosis patients having values less than 5. However, only 2 of these had lower readings than the highest IgM values produced by 63 of the 64 tested control sera.

A second serum specimen was obtained from 8 of the patients with acute brucellosis 1 to 8 months after the original specimen was taken. In specimens from 7 patients, the ELISA values obtained with the two sera were similarly elevated. However, in patient 16 the readings with the second specimen were elevated, whereas the ELISA values (as well as the agglutination titres) produced with the admission serum were relatively low.

3 of the patients who did not have symptoms of acute

TABLE I—AGGLUTINATION TITRES, BLOOD-CULTURE RESULTS, AND ELISA VALUES IN PATIENTS WITH ACUTE BRUCELLOSIS

Patient no.	*Brucella* blood culture	Agglutination titre	ELISA values		
			IgG + IgM + IgA	IgG	IgM
1	+	1280	10·5	8·1	7·8
2	0	640	9·8	9·3	3·6
3	+	2560	11·0	9·7	5·5
4	+	640	9·1	9·3	4·3
5	+	80	17·5	10·4	3·7
6	+	320	7·8	10·5	6·3
7	+	640	9·7	9·2	5·8
8	+	80	12·5	9·9	4·2
9	0	1280	12·5	10·2	9·5
10	+	2560	9·3	9·8	10·0
11	+	640	7·7	7·6	4·1
12	+	2560	7·5	8·0	4·1
13	+	320	7·0	7·0	5·7
14	+	320	8·9	8·5	4·8
15	+	640	9·9	8·5	7·7
+ 1 mo			8·5	8·3	7·8
16	+	80	4·8	6·8	2·8
+ 1 mo		1280	8·2	7·5	7·2
17	+	1280	10·0	10·0	4·4
+ 1 mo		2560	9·7	10·0	4·6
18	0	320	16·3	9·6	5·6
+ 4 mo		320	16·6	10·3	5·1
19	+	640	8·3	9·9	7·6
+ 1 mo			9·4	8·9	7·8
20	+	1280	8·0	10·2	3·8
+ 8 mo			11·0	8·9	3·0
21	+	2560	8·6	10·6	9·1
+ 7 mo			9·9	8·9	7·4
22	+	1280	9·9	9·6	6·0
+ 6 mo			10·6	11·6	5·6

brucellosis had elevated IgG. Patient 23 had low-grade fever and a history indicative of chronic brucellosis; patient 24 had a history of confirmed acute brucellosis; and patient 25 had schistosomiasis and *Salmonella paratyphi* A infections but no evidence of brucellosis. These 3 patients had agglutination titres of 1:80. Clinical data were not available for patient 26, which is unfortunate because he had an agglutination titre of 1:2560 and high IgG and IgM values, and most likely had acute brucellosis.

The IgG and total ELISA values for individual serum specimens were usually similar, and those that had high IgM readings always had high IgG and total ELISA values. All of the patients with acute brucellosis had agglutination titres of at least 1:80, the median value being 1:640. There was a poor correlation between IgM values and agglutination titres ($r = 0 \cdot 48$).

TABLE II—AGGLUTINATION TITRES AND ELISA VALUES IN FEBRILE PATIENTS NOT SUSPECTED OF HAVING ACUTE BRUCELLOSIS

Patient no.	Agglutination titre	ELISA values		
		IgG + IgM + IgA	IgG	IgM
23*	80	5·4	8·4	2·0
24†	80	7·3	7·3	3·1
25‡	80	9·8	8·7	3·8
26	2560	7·2	6·7	6·9
27–87	<40	<3·0	2·3§	1·6¶
88	40	4·9	ND	ND
89–90	80	<3·0	ND	ND
91–249	<20‖	<3·0	ND	ND
251–409	ND	<3·0	ND	ND

ND = not done.
*Patient had clinical symptoms and history compatible with chronic brucellosis.
†Patient had clinical symptoms of brucellosis and an agglutination titre of 1:1280 in 1968.
‡Patient had *Salmonella paratyphi* A cultured from the blood plus schistosomiasis.
§Mean value of 2·3, range 1·1–4·8.
¶Mean value of 1·6, range 1·4–3·6.
‖By slide agglutination.

Discussion

Pellerin et al.,[8] using an assay for total human *Brucella* antibodies, found ELISA to be more effective than other methods for diagnosis of brucellosis. With a similar ELISA we found values greater than 7 in all patients with acute brucellosis, whereas all but 5 of the almost 400 control patients had readings below 3. Such differences can easily be seen with the naked eye, making the ELISA for total *Brucella* antibodies an excellent method for screening human sera, especially in developing countries where this disease is still an important public-health problem.

Our data are also in general agreement with the results of previous studies on humoral response in human brucellosis which demonstrate that elevated IgM anti-*Brucella* antibody levels are consistent with acute disease, whereas high IgG, in the absence of IgM, suggests a chronic infection.

Magee reported elevated IgM levels in his 3 patients with acute brucellosis but in none of the sera from patients with chronic brucellosis or controls.[6] We also found high IgM levels exclusively in patients with acute disease. However, several of our culture-positive patients had IgM values that were marginal in that they were in the same range as the highest of the controls. When IgM values were measured at higher serum dilutions (data not shown) to determine if these

low levels were due to a prozone effect caused by high concentrations of IgG antibodies, increases in the levels of IgM immunoglobulins could not be detected. In Magee's study, the acute patient who had not been treated before hospital admission had a much higher IgM value than the 2 who had received antibiotics. Considering the overuse of antibiotics in Egypt,[9] this could have been a factor with the IgM levels in our acute patient group.

In their elegant studies on 2-mercaptoethanol (2-ME)-sensitive (IgM) and 2-ME-resistant (IgG) antibody response to *Brucella* infections, Reddin et al.[1] found that patients with acute disease had elevated macroglobulin and microglobulin levels but that there was a striking fall in the latter within 8 months. Although we also found elevated IgM and IgG levels in our large group of acute patients, we did not detect a decrease in either immunoglobulin class of *Brucella* antibodies in treated patients over an 8-month period. Our data are also not in complete agreement with those of Parratt et al.,[2] who found that some patients had elevations of IgM *Brucella* antibodies only. These inconsistencies may, to some extent, be due to differences in methods. However, they more likely reflect differences in the patient populations studied: the 2 previous reports[1,2] were from developed countries, where brucellosis is generally diagnosed quickly and treated with antibiotics for long periods thereby making acute and chronic brucellosis two distinct immunological entities. Our patients, on the other hand, were from the rural Delta of Egypt, where immediate diagnosis and adequate treatment are seldom possible. Many of the patients that we describe as having acute brucellosis because of their clinical presentations and (in most instances) positive blood cultures may instead have a long-term illness which was never adequately treated and thus they would have immunological presentations consistent with both acute and chronic disease.

Only 3 subjects had had high IgG and low IgM levels of *Brucella* antibodies. 1 (no. 24) had acute brucellosis 12 years previously but no symptoms currently that are suggestive of an active infection. Another (no. 23) presented with a history and symptoms that were consistent with chronic brucellosis. The third (no. 25) had no history or clinical presentation indicative of brucellosis, suggesting that some chronic infections are subclinical. A strong IgG response with little or no IgM antibody production has also been reported for secondary rickettsial[10,11] and typhoid[12] infections and is probably a reflection of the population of B lymphocytes that persists after an active infection.

The opinions and assertions contained herein are those of the authors and are not to be construed as official or as reflecting the views of the Department of the Navy or the Naval Service at large.

We thank A. S. Diab for his excellent technical assistance.

This work was supported by the Naval Medical Research and Development Command, National Naval Medical Center, Bethesda, Maryland, work unit no. M0095-PN-002-5020.

Requests for reprints should be addressed to the Publications Office, NAMRU-3, c/o U.S. Embassy, Cairo, Egypt. Correspondence should be addressed to J. E. S., Naval Biosciences Laboratory, Naval Supply Center, Oakland, California 94625, U.S.A.

REFERENCES

1. Reddin JL, Anderson RK, Jenness R, Spink W. Significance of 7S and macroglobulin brucella agglutinins in human brucellosis. *N Engl J Med* 1965; **272:** 1263–68.
2. Parratt D, Nielsen KH, White RG, Payne DJH. Radioimmunoassay of IgM, IgG, and IgA brucella antibodies. *Lancet* 1977; i: 1075–78.
3. Macdonald A, Elmslie WH. Serological investigations in suspected brucellosis. *Lancet* 1967; i: 380–82.
4. Kerr WR, Coghlan JD, Payne KJH, Robertson I. The laboratory diagnosis of chronic brucellosis. *Lancet* 1966; ii: 1181–83.
5. Diaz R, Maravi-Poma E, Rivero A. Comparison of counter-immunoelectrophoresis with other serological tests in the diagnosis of human brucellosis. *Bull WHO* 1976; **53:** 417–24.
6. Magee JT. An enzyme-labelled immunosorbent assay for *Brucella abortus* antibodies. *J Med Microbiol* 1980; **13:** 167–72.
7. Voller A, Bartlett A, Bidwell OE. Enzyme immunoassays with special reference to ELISA techniques. *J Clin Path* 1978; **31:** 507–20.
8. Pellerin JL, Geral MF, Lautie R. Le test immuno-enzymatique E.I.E.S.A. dans le diagnostic serologique de la brucellose humanine. *Rec Med Vet* 1980; **131:** 741–66.
9. Farid Z, Miner WF, Hassan A, Trabolsi B. Misuse of antibiotics. *N Engl J Med* 1975; **292:** 216.
10. Murray ES, O'Connor JM, Gaon JA. Serologic studies of primary epidemic typhus and recrudescent typhus (Brill-Zinsser disease) II. Differences in immunoelectrophoretic patterns, response to 2-mercaptoethanol and relationships to 19 S and 7S antibodies. *J Immunol* 1965; **94:** 734–40.
11. Bourgeois AL, Olson JG, Fang RCY, et al. Humoral and cellular responses in human scrub typhus. *Am J Trop Med Hyg* (in press).
12. Chernokhvostova E, Luxemburg KI, Starshinova V, Andreeva N, German G. Study on the production of IgG-, IgA- and IgM-antibodies to somatic antigens of *Salmonella typhi* in humans. *Clin Exp Immunol* 1969; **4:** 407–21.